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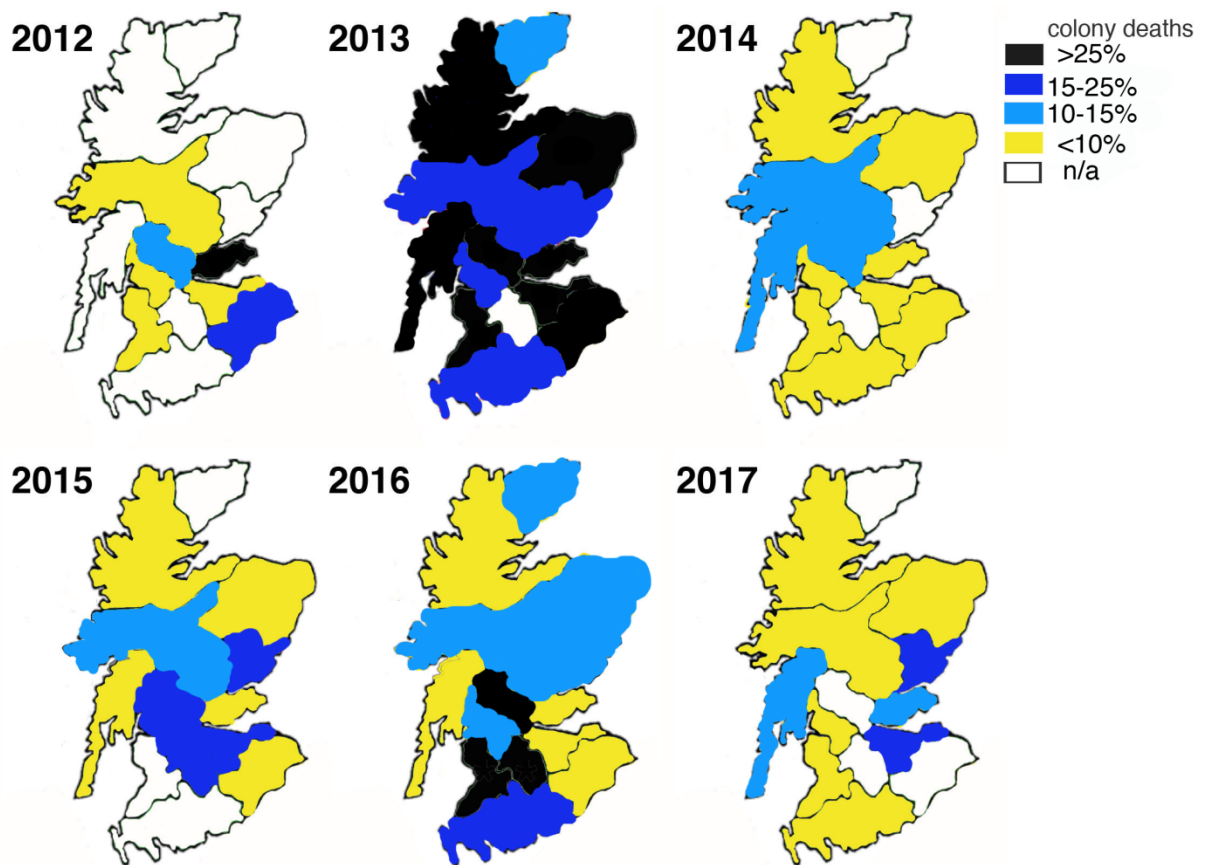
## Honeybee colony losses and threats in Scotland. (2012-2017)

We present here the summary of the findings on honeybee colony failures over winter in Scotland. These data were kindly provided to the University of Dundee by participating members of the *Scottish Beekeepers Association* (SBA). The great news in 2017 is that it has been another successful year for honeybee overwintering in Scotland, with average colony losses of only 9.4% (Table 1). This study was initiated due to a period of high colony losses being reported between 2007 - 2011 (17.5 - 21.4% colony deaths)<sup>1</sup>. Our study indicates that the high death rates in Scotland ('normal' failure rates are about 10%) continued (from 2007) to 2013, but decreased thereafter (Table 1).

Postcode	2012	2013	2014	2015	2016	2017	Area average (2012-17)
ML	-	-	-	21.4	31.3	-	26.4
FK	13.3	35.7	10.5	17.6	28.2	-	21.1
DD	-	18.2	-	19.0	12	25.0	18.6
KA	3.9	42.1	2.6	-	38.1	3.7	18.1
KY	30.9	30.3	9.7	7.4	8.3	-	16.6
EH	5.8	32.9	9.3	16.5	7.2	17.3	14.8
<b>Year average</b>	<b>20.0</b>	<b>24.5</b>	<b>8.4</b>	<b>9.3</b>	<b>13.6</b>	<b>9.4</b>	<b>14.2</b>
AB	-	34.4	9.4	4.7	11.7	7.5	13.5
PA	-	29.4	11.8	5.2	4.8	14.0	13.0
TD	18.7	25.0	8.2	6.1	4.2	-	12.4
KW	-	10.5	-	-	11.8	-	11.2
PH	6.5	19.2	12.5	10.8	13.0	4.2	11.0
DG	-	17.7	4.4	-	16.2	5.4	10.9
G	5.6	16.7	5.6	15.4	12.5	4.6	10.1
IV/HS	-	25.9	3	2.9	4.7	6.2	8.5

**Table 1.** Percentage overwintering failures (2012-2017) by postcode region. Regions have been ranked by their average failure rate over the six years (right hand column) and related to the average. Note: The average failure rate is derived from the raw data, not the average of year averages. The shaded boxes indicate where losses are significantly greater (>15% = blue and >25% = black) than an acceptable failure rate of 10%.

Our findings demonstrate a high variability in colony deaths between areas, with very low rates of colony losses found in remote areas (eg. Inverness (IV) & the Outer Hebrides (HS)). To illustrate the variation in losses in each postcode area<sup>(a)</sup> we have produced a series of maps to illustrate the colony failure rates in Scotland over the past 6 years (Figure 1). High colony losses are indicated in light blue (10-15% losses), dark blue (15-25% losses) and black (>25% losses). Clearly, 2013 was a bad year for the whole of Scotland as well as the rest of the UK.



**Figure 1.** Map indicating the loss of colonies in each county (2012-16). Counties in white had too few apiary returns (< 5) to include in the analysis, but the data did contribute towards the overall assessment (see table 2).

When considering the problems, it is important to remember that there are two things that bees do not like, the wind and the rain, and Scotland has both in abundance. The key anthropogenic threats to UK honeybees include the loss of wildflowers and hedgerows, pesticide exposure and imported threats such as the ‘blood’-sucking *Varroa* mite and the gut parasite *Nosema ceranae*. The future holds new additional threats such as the Small Hive Beetle (already in Europe) and the Asian Hornet (already in the UK). In addition, imported bumblebees for poly tunnel pollination of crops and honeybees to replace lost colonies or replace old queens may bring new disease threats.

### Neonicotinoids

The neonicotinoids were hailed as a breakthrough in insecticide design, having low toxicity to vertebrates and high efficacy on target pest species. Importantly, when used as seed coatings, their use was thought to be highly targeted to the crops. By the time the seed-treated crop flowered (and attracted bees), neonicotinoid levels were negligible, just a few parts per billion, which is equivalent ratio to one second in 10 years. Unfortunately, although this is too low to kill bees, it is sufficient to block brain cell function.

The use of the three key neonicotinoids, imidacloprid, clothianidin and thiamethoxam were substantially increased since 2000, with clothianidin and thiamethoxam being introduced in 2006 and 2008, respectively. Their introduction coincides with the time when consistently

high bee losses began. These three neonicotinoids were banned for use on bee-visited crops in the EU in 2013. So, have bees recovered since their restriction?

When considering the impact of the EU ban, three important points need to be considered. Firstly, the neonicotinoids may be replaced with alternative insecticides, so any benefits to bees cannot be assured. Secondly, neonicotinoid use on other crops is not affected by the ban. Thirdly, neonicotinoids appear to be more persistent in the soil than previously indicated and have been found to move to adjacent wildflowers and hedgerows<sup>2,3</sup> and are washed into our waterways<sup>(4)</sup>. Given their continued substantial use on other crops, exposure to bees is likely to continue. However, we do not know if the use of other insecticides has increased and by how much neonicotinoid has been used as no government survey has yet been done since the ban has been effective<sup>b</sup>. The first data will be released by *Science & Advice for Scottish Agriculture (SASA)* on 31<sup>st</sup> October 2017.

In our survey of the impact of exposure to neonicotinoids, we focused on a single major source of exposure to honeybees, seed-coating treatment of oilseed rape (OSR). Previous surveys<sup>(c)</sup> detected a correlation between bees foraging on OSR and overwintering failures<sup>1</sup>. In 2012, our survey also detected this correlation. However, in 2013 and 2014, when OSR crops were also treated with neonicotinoids, no effect was evident. Therefore, direct exposure to a treated crop may not be a reliable indicator of colony failures. However, we should also consider indirect exposure that occurs via non-treated plants such as wildflowers. In contrast to direct exposure from crops, indirect exposure in intensively arable landscapes is prolonged, occurring throughout the foraging season. Therefore, actual exposure to neonicotinoids is unknown as information on the local application of neonicotinoids, or any other pesticide, is not available.

To understand how the neonicotinoids could affect bees, we need to consider their main mode of action. Neonicotinoids are designed as neurotoxins to target the brains of insect pests, but they also work on the brains of other insects, including bees. In bees, we know that they cause brain cell inactivation after exposure to normal field levels. This brain cell deficit explains why, in both honeybees and bumblebees, treated bees have poorer olfactory learning ability – the ability to learn floral scent as an indicator of a nectar or pollen reward. Interestingly, bumblebee learning is not affected by clothianidin, whereas honeybee learning is, indicating that not all neonicotinoids are the same and that species vulnerability to individual neonicotinoids differ<sup>6-7</sup>. Exposed vulnerable bees become poor foragers<sup>8</sup>, taking longer to find less food. If many bees are affected, the colony struggles to feed its young and begins to weaken. Several studies have demonstrated that neonicotinoid-treated bumblebee colonies are weaker when exposed to imidacloprid or thiamethoxam, but not clothianidin<sup>8-11</sup>. For the larger honeybee colonies, the impact on the colony may take longer to show.

The exposure to neonicotinoids may be magnified by preference seeking behaviour<sup>12</sup>, whereby bees seek out contaminated sources. This is akin to smokers seeking nicotine in cigarettes and neonicotinoids (neo = new, nicotinoid = nicotine-like) are modified forms of nicotine to be more effective on insects. A compounding effect occurs due to molecular adaptations when brain cells are exposed chronically to neonicotinoids, as an increase in the

expression of the target receptor leads to brain cells becoming more sensitive, and bees become more vulnerable, to the effects of neonicotinoids<sup>10</sup>.

Despite the many laboratory studies that demonstrate a direct cause and effect of neonicotinoids, contradictory evidence has come from some field trials. However, these studies lack replication (very few samples) and their analyses heavily criticised by experts. There are several problems with field trials and the use of honeybee colonies to indicate risk to all bees and other insect pollinators. Firstly, honeybee colonies are big (up to 60,000 bees) and so have a strong buffering capacity that can transiently mask any decrease in worker performance. Secondly, arable environments are highly complex and no two are the same. For example, there are unknown levels of other pesticides present, as this information is not made available, habitats vary in quality, there are unknown bee diseases/parasites present, and adverse local weather conditions during peak foraging opportunities are not recorded. Thirdly, and most importantly, colonies are only placed on site for a short dose of exposure, before being returned to protected areas of low pesticide use and excellent natural forage. Therefore, the best possible evidence that any field trial may generate is a correlation with a known factor, where the impact requires only a short exposure and dominates all other unknown confounding factors. This has been a problem in the past, as other neonicotinoids have turned up unexpectedly, making interpretation of the data convoluted and invalid. Regardless, everything else accepted, correlation is not causation. Robust evidence requires carefully controlled experimentation to demonstrate cause and effect.

When such laboratory studies have been done, cause and effect has been confirmed. The evidence is clear, there is a negative impact on individual bees<sup>4-11</sup>, colony development<sup>8-11</sup> and overwintering<sup>13-14</sup>. We realise now that the exposure period to neonicotinoids is extended by translocation in the soil to non-treated plants<sup>2,3</sup>. Therefore, exposure to insects living within an intensively managed arable setting is chronic, not just acute when treated crops are in flower. Chronic exposure is a very different problem for our bees as recovery is not possible.

In support of existence of long-term chronic exposure, a recent study has identified the presence of several neonicotinoids in honey<sup>15</sup>. This study analysed 198 samples of local honey gathered from across the globe and neonicotinoids were found (at neuroactive levels for bees) in 86% (North America), 80% (Asia) and 79% (European) of honey samples, with the lowest frequency being detected in South America (57%)<sup>15</sup>. Therefore, this study provides clear evidence that chronic exposure of bees to neonicotinoids does occur, and on a global scale.

Although the accumulation of neonicotinoids from the environment into honey is alarming, it does also provide a new tool to monitor environmental contamination, not just of neonicotinoids, but of other chemicals too<sup>16</sup>. Better than this would be direct access to the data on actual local pesticide use. Although this is required under EU and US law, the data are not gathered and so we want learn from past mistakes of chronic exposure or unknown chemical cocktail effects. Recently, the Chief Scientific Adviser to DEFRA has advocated the need for better monitoring of pesticide use (which they termed 'pesticidovigilance') and highlighted our lack of understanding of the risk from the current industrialised scale of

pesticide use in the UK<sup>17</sup>. Until this proposal becomes reality, a correlation of local honey contamination with local environmental problems or chronic disease rates, may provide a new insight into the impact of pesticides on our environment and health.

The monitoring of honey contamination has another, more immediate, use – to assess the effectiveness of the 2013 EU moratorium, which aimed to reduce the exposure of bees to neonicotinoids. As most (~70%) uses of neonicotinoids are unaffected by this moratorium, and we know that they can be taken up by wildflowers<sup>2-4</sup>, has exposure to bees been eliminated? Therefore, an analysis of neonicotinoids in EU honey since the moratorium became effective would indicate the degree of success of a partial ban in reducing bee's exposure to neonicotinoids and so identify whether a full ban is required.

Finally, although the negative impact of neonicotinoids on bees is now widely accepted, it is important to consider how the neonicotinoids exert their negative effects, in order that we may understand how to mitigate against these effects. An important clue is provided by beekeepers living away from intensively managed arable crops, who move their bees to OSR yet do not experience any problems with colony performance or survival. An explanation may be provided by the fact that normal neonicotinoid exposure is not acutely toxic, but may cause sublethal brain effects and disrupt their ability to forage. During exposure, a large field of contaminated OSR nectar is freely available, but these short-term deficits can be buffered by the large honeybee colonies. When the colony is returned to their 'home' landscape, the exposure to neonicotinoids is terminated and individual bees recover. This scenario is supported by the fact that caged laboratory colonies of bumblebees exposed to neonicotinoids do not experience any deficits, because they are not required to forage to feed their brood, sugar and pollen being provided directly in the nest<sup>18</sup>. In contrast, bees restricted to intensively managed areas of arable crops experience prolonged exposure to neonicotinoids and must therefore forage in a difficult habitat, depleted of natural foraging opportunities (eg. wildflowers), while their foraging skills are handicapped by neonicotinoid intoxication.

## Varroa

Another major threat to honeybee colonies is the imported 'blood'-sucking parasite, the varroa mite (*Varroa destructor*) and beekeepers across Scotland are forced to use a range of different approaches (including pesticides) to control mite numbers within their bee hives.



**Figure 2.** The varroa mite (*Varroa destructor*) seen from below (left) and when feeding on a honeybee (right). Pictures by C.N. Connolly and T. Dixon (University of Dundee).

### Impact of colony treatment:

Early in this project we assessed the possible impact of different anti-varroa treatments used on the overwintering success of the treated colonies. Our findings demonstrated that only formic acid and Apistan appeared to decrease the overwinter survival rate, with failure rates in 2013 of 39% (formic acid) and 36.7% (Apistan), compared to the average of all treatments (24.6%). The possible increase for formic acid appeared to reflect damage due to high varroa infestation levels. For Apistan, increased colony failures may be due to the development of Apistan-resistance of the varroa mites. In support of this possibility, when other treatments were used in addition to Apistan, overwintering losses were normal for that year.

### Impact of infestation:

To address the impact of varroa infestation on colony overwinter survival rates we asked beekeepers to record the level of infestation in their colonies and report on their colony losses after winter (2012-2017). Colonies were reported as being 'heavy' if infestation was a problem, 'medium' if the infestation was under control and 'light' when, although present, did not require treatment to protect the colony.

Year	Neonicotinoid use		% colony failures						
	*Non-OSR (Kg)	*OSR (Kg)	overall losses	OSR		Varroa level			
				-	+	High	Medium	Light	None
2012	1097	509	20	15.9	30.3	-	-	-	-
2013	1086	509	24.5	27	21.3	24.7	28.8	35.6	12.6
2014	1360	380	8.3	9	7.3	5.7	12.7	5.5	2.1
2015	?	0	9.3	9.8	7.9	33.3	11.2	3.4	1.8
2016	?	0	13.6	13.8	13	4.4	14	18	12.2
2017	?	0	9.4	8.8	11.3	14.8	9.1	7.8	15.3
			2012-2017	15.7	14.6	16	17.9	11.5	10.4

**Table 2.** Pesticide use data provided by FERA derives from SASA (collected every 2 years). The last pesticide use data provided is from 2014. There is no data to assess subsequent neonicotinoid use, or any compensatory use of other insecticides since the moratorium began in 2014. Red shaded data indicates a lack of effect of direct exposure from nearby OSR crops. Blue shaded data suggests a negligible effect of Varroa infestation. \*Data from FERA's website (<http://pusstats.fera.defra.gov.uk/>) collected in 2012 and 2014 and extrapolated to 2013. Note: The non-OSR use is predominantly (99%) for use on cereal crops and largely (97%) represents the use of clothianidin.

Here we pool the data from the last 5 years' reports (2013-17, Table 2) into categories based on the reported level of varroa infestation. The number of colonies and apiaries were: high (39 apiaries with 206 colonies), medium (513 apiaries with 2,330 colonies), low (170 apiaries with 923 colonies) and no (195 apiaries with 337 colonies) varroa infestation. Therefore, over 90% of honeybee colonies were infested. The results indicate that there was



no clear overall impact of varroa load (heavy, medium and light) on overwintering survival (Table 2, blue highlighted boxes). Where Varroa is reported to be absent, colonies were largely restricted to remote areas where there is also very little (if any) exposure to pesticides.

This evidence does not mean that varroa is not a major threat to bees, but that this threat is being contained by Scottish beekeeper vigilance and treatment. An important caveat to this risk, is the fact that the Varroa mites transmit hybrid viruses, with high toxicity, and this secondary risk requires monitoring, but was not studied here.

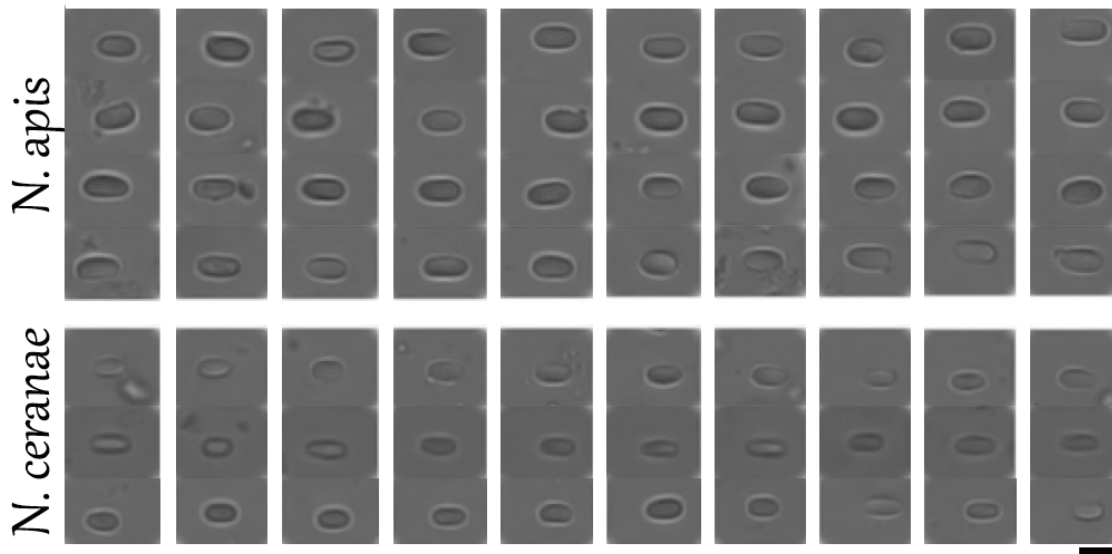
### ***Nosema ceranae***

The Western honeybee, *Apis mellifera*, is endemically infected with a unicellular internal parasite, *Nosema apis*, that multiplies in the bee gut and spreads by faecal contamination. In recent years, a related parasite, *Nosema ceranae*, which normally infects the Eastern honeybee, *Apis ceranae*, has been imported from Asia and now infects our Western honeybee and bumblebees in the UK. The risk from *Nosema ceranae* to our honeybees is unclear but it has been spreading throughout world populations of Western honeybees, but the picture in Scotland was unknown previously.

An ambitious project initiated by the University of Dundee, Science & Advice for Scottish Agriculture (SASA) involved the training of a team of beekeepers in the molecular skills required to assess the landscape of Nosema in honeybees across Scotland. A team of very talented beekeepers rose to the challenge of distinguishing between *Nosema ceranae* from *Nosema apis* and mapping their presence in Scotland. Many local associations did the primary microscopic screening of hundreds of colonies for the presence of Nosema spores in their region and samples found to possess spores were forwarded for molecular analysis. If the team were successful in distinguishing between these two species by microscopy alone, their methodology could be adopted by beekeepers across the globe, making it feasible for the spread of *Nosema ceranae* to be monitored more easily.

Following the primary screening by local associations, 75 positive samples were analysed by molecular (polymerase chain reaction) and microscopic approaches and the team was completely successful in confirming that microscopy alone can distinguish between *Nosema apis* and *Nosema ceranae*, based on the size of spores present in bee guts. Importantly, the SBA team's evidence demonstrated that *Nosema ceranae* had spread across Scotland quickly, replacing our endemic strain, *Nosema apis*, as the predominant strain of Nosema. To date, no evidence of damage to Scottish honeybee colonies is evident and the impact of widespread infection by *N. ceranae* is unclear.





**Figure 3. Light microscopy of *Nosema* spores (x400 magnification) from honeybee guts.** Spores from *N. apis* (top) and *N. ceranae* (bottom). Can you spot the difference? Scale bar = 5 microns.

### Summary

Although the results of this six-year survey have not been definitive in terms of identifying a cause(s) of honeybee colony losses in Scotland, this is not unexpected given the vast complexity of the farmed environment, where multiple new threats exist. Nevertheless, we have excluded the key threats (*Varroa* infestation, direct exposure to neonicotinoid-treated crops and *Nosema ceranae* infection) as being the sole driver(s) of honeybee losses. However, indirect risks, such as a secondary (and chronic) exposure to neonicotinoids or the transmission of viruses by *Varroa*, may yet prove to be major threats to honeybees. Further complexity exists, as it is known that multiple known stressors can interact with each other to increase further the risk to bees.

An important take home message is that neonicotinoid-induced deficits manifest as poor foraging ability. Therefore, by providing a much richer habitat of alternative, non-contaminated, bee forage in our gardens, all insect pollinators may find foraging less difficult, their exposure to the neonicotinoid-treated crop (or other pesticides) would be reduced and they could recover from any detrimental effects of prior exposure. For beekeepers, it is important to be vigilant in the control of the *Varroa* mite and one should obtain honeybees (including queens) locally, as this will restrict the spread of global bee diseases and parasites. Farmers could benefit our bees by providing permanent set-aside areas where native plants could establish and by reducing pesticide use. As a result, their land may recover some of its original value as an ecosystem, including the multiple insect pollinators, pest predators and soil organisms. Together, they will repay this help by improving the quality, yield and sustainability of insect pollinated crops.

#### Notes:

- a. The exact location of apiaries is not considered in this report – a more detailed geographical assessment is under way.
- b. Pesticide usage survey are only conducted every other year. The last survey was done in 2014, where the 2013 use of neonicotinoid-coated OSR seed was included. The next survey due is 2016 and the results will be available on 31<sup>st</sup> October 2017.
- c. Colony death rates revealed a two-fold increase failure rate when bees foraged on OSR in the previous summer. Losses were, 2006-7 (29% vs 15%), 2007-8 (35% vs 19%) and 2010-11 (30% vs 15%). Study by Magnus Peterson & Alison Gray, University of Strathclyde.

#### References.

<sup>1</sup> Magnus Peterson & Alison Gray, 'Investigation of winter loss rates'. The Scottish Beekeeper, August 2012

<sup>2</sup> <http://science.sciencemag.org/content/356/6345/1395.full>

<sup>3</sup> <https://doi.org/10.1016/j.scitotenv.2016.05.065>

<sup>4</sup> <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5533829/#!po=21.0000>

<sup>5</sup> <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3621900/>

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<sup>8</sup> <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3495159/>

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<sup>10</sup> <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4849185/>

<sup>11</sup> <http://science.sciencemag.org/content/336/6079/351.long>

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<sup>13</sup> <http://science.sciencemag.org/content/356/6345/1393>

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<sup>15</sup> <http://science.sciencemag.org/content/358/6359/109>

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<sup>18</sup> <https://www.ncbi.nlm.nih.gov/pubmed/28808317>

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